

WHAT IS CLAIMED IS:

1. A method for identifying DNA mutation using microwells which comprises the steps of:

5 (i) preparing amplified biotinylated DNA fragments of a portion of nucleotide sequence to be identified by PCR using a biotin-bound primer;

(ii) preparing a probe comprising normal sequence corresponding to the DNA sequence to be identified;

10 (iii) affixing the probe prepared in Step(ii) to the amine group of microwell;

(iv) adding biotinylated DNA fragments prepared in Step(i) to the probe-affixed microwell;

15 (v) adding a streptavidin-linked degradation enzyme to the microwell in order to bind the degradation enzyme to biotin moiety of the probe-captured sample DNA fragment; and,

20 (vi) adding a substrate to be reacted with the degradation enzyme and detecting the color or absorbance change caused by degradation of the substrate.

25 2. The method for identifying DNA mutation of claim 1, wherein the probe is a nucleotide sequence consisting of more than 10 nucleotides and containing a phosphate moiety at 5' end.

30 3. The method for identifying DNA mutation of claim 1, wherein Step(iii) comprises adding single-stranded probe to the microwell, adding catalysts in order to bind the phosphate moiety of the single-stranded probe to the amine group of microwell, and washing the microwell.

35 4. The method for identifying DNA mutation of claim 3, wherein the catalysts are ice-cold solutions of 10mM 1-methylimidazole, pH 7.0 and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC), pH 7.0.

5. The method for identifying DNA mutation of claim 3, wherein the washing is performed by employing 0.4M NaOH/0.25% Tween-20 solution.

5 6. The method for identifying DNA mutation of claim 1, wherein Step(iv) comprises binding single-stranded DNA fragments obtained in Step(i) to the probe of microwell, removing the residual DNA fragments, and washing the microwell.

10 7. The method for identifying DNA mutation of claim 6, further comprising pretreatment of the microwell with a solution containing dH₂O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml) at 50°C for 20 minutes before adding the single-stranded DNA fragments obtained in Step(i) to the microwell.

15 8. The method for identifying DNA mutation of claim 6, wherein binding the DNA fragments obtained in Step(i) to the probe occurs in a solution containing dH₂O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml).

20 9. The method for identifying DNA mutation of claim 6, wherein the washing is performed by employing 0.5xSSC/0.1% Tween-20 solution.

25 10. The method for identifying DNA mutation of claim 1, wherein Step(v) comprises the first washing of the microwell, introducing streptavidin-linked degradation enzyme to the microwell for binding of the enzyme with biotin, removing the residual reaction mixture, and the second washing of the microwell.

30 11. The method for identifying DNA mutation of claim 10, wherein the first washing is performed by employing a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl/0.1% Tween-20.

12. The method for identifying DNA mutation of claim 10, wherein the streptavidin-linked degradation enzyme is streptavidin-alkaline phosphatase dissolved in a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl.

13. The method for identifying DNA mutation of claim 10, wherein the second washing comprises treatment of the microwell with a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl/0.1% Tween-20 at 60°C for 10 minutes.

14. The method for identifying DNA mutation of claim 1, wherein the substrate to be reacted with streptavidin-linked degradation enzyme is a synthetic peptide showing color or absorbance change during the degradation.

15. The method for identifying DNA mutation of claim 14, wherein the substrate is pNPP(p-nitrophenyl phosphate) provided that the streptavidin-linked degradation enzyme of streptavidin-alkaline phosphatase is employed.

16. The method for identifying DNA mutation of claim 1, wherein the color change is detected with naked eyes.

17. The method for identifying DNA mutation of claim 1, wherein the absorbance is measured by employing an ELISA reader.

18. A kit for identifying DNA mutation which comprises:

- (i) a microwell whose inside has amine group;
- (ii) 10mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC), pH 7.0 and 10mM 1-methylimidazole, pH 7.0;
- (iii) 0.4M NaOH/0.25% Tween-20 solution;
- (iv) a solution containing dH₂O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml);

- (v) 0.5xSSC/0.1% Tween-20 solution;
- (vi) streptavidin-alkaline phosphatase;
- (vii) 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl;
- 5 (viii) 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl/0.1% Tween-20; and,
- (ix) pNPP(p-nitrophenyl phosphate).